whose blood expression was altered compared to healthy subjects. In OA, 9 miRNA were up-regulated (including miR-228, miR-574-3p, miR-597) and 9 miRNAs were down-regulated (including miR-150, miR-222, miR-363 and miR-423). None of miRNAs in OA is common with those we found in RA. Potential targets of miRNAs, specifically expressed in severe knee osteoarthritis, appears to be largely involved in the Wnt signaling pathway. In contrast, the miRNAs expressed differently in the blood of our RA patients seem to target the elements of the signaling pathway MAP kinase.
Conclusions: Our results suggest that miRNAs may constitute new biomarkers potential diagnostic interest. In addition, miRNAs could be involved in the pathogenesis of OA. Further work is ongoing in order to assess the pathophysiological and the functional role of these miRNAs in OA.

069

STATIN USE IS ASSOCIATED WITH REDUCED INCIDENCE AND PROGRESSION OF KNEE OSTEOARTHRITIS

S. Clockaerts[1,2], B. Stricker[2], Y.M. Bastiaansen-Jenniskens[2], F. Van Glabbeek[1], J.B. Van Meurs[2], J.A. Verhaar[2], A. Hofman[2], G.V. Van Osch[2], S.M. Bierma-Zeinstra[2]
[1]Univ. of Antwerp, Edegem, Antwerp, Belgium; [2]Erasmus MC, Univ. Med. Ctr., Rotterdam, Netherlands

Purpose: Besides biomechanical and genetic alterations, the pathogenesis of osteoarthritis (OA) may involve inflammation, vascular alterations and dysregulation of lipid metabolism. Statins are drugs capable of modulating many of these different mechanisms and therefore may have the potential to act as disease modifying drugs for osteoarthritis. In this study we hypothesized that statins decrease incidence and progression of knee and hip OA. To test this hypothesis, we used a large population cohort study.
Methods: 2974 subjects of the Rotterdam Study (a population-based cohort study), aged 55 years and older were included in this study. X-rays of the knee and the hip were obtained at baseline and follow up (mean follow up 6.3 years), and were scored with the Kellgren & Lawrence score (0=absent OA, 1=doubtful OA, 2=mild OA, 3=moderate OA, 4=severe OA, 5= prosthesis). Incidence of OA was defined as a Kellgren & Lawrence score of 0 or 1 and a score of 2 or more at follow up. Progression of OA was specified as a Kellgren & Lawrence score of 1, 2 or 3 and increase of 1 or more. Information on statin use was obtained from detailed computerized pharmacy data. Use of statins was defined as the daily use of 50% or more of recommended dose and this for a period of 100 days or more. Data on potential confounding variables such as age, gender, body mass index, diabetes mellitus, arterial hypertension, peripheral artery disease, bone mineral density and total cholesterol level were collected. A multivariate logistic regression model adjusting for confounding variables was fitted to calculate odds ratios with confidence intervals. Correlations between right and left joints were accounted for with generalized estimating equations.
Results: Osteoarthritis (Kellgren & Lawrence score 2 or more) was present in 546 knees and 323 hips at baseline and in 696 knees and 521 hips at follow up in 1277 men and 1697 women. Overall, 13.2% of subjects were defined as statin users. The adjusted odds ratios for incidence of knee OA in users of statins was 0.40 (95% CI 0.20 - 0.80, p=0.01) and for progression of knee OA 0.47 (95% CI 0.25 - 0.87, p=0.02). The use of statins was neither associated with incidence of hip OA (adjusted odds ratio 0.85, 95% CI 0.54 - 1.34, p=0.48), nor with progression of hip OA (adjusted odds ratio 1.13, 95% CI 0.71 - 1.81, p=0.61).
Conclusions: Statin use is associated with a reduction in incidence and progression of knee osteoarthritis. Randomized clinical trials in a population at risk are needed to examine whether statins could be useful as a treatment for knee OA.

070

EIGHTY-SEVEN PERCENT OF 66 ADULT PATIENTS WITH ADVANCED OSTEOARTHRITIS OF THE KNEE SUBJECTED TO TREATMENT WITH INTRA-ARTICULAR INJECTIONS OF HUMAN GROWTH HORMONE AVOID TOTAL KNEE ARTHROPLASTY

A.R. Dunn
Mt. Sinai Med. Ctr., Miami Beach, FL

Purpose: Intra-articular injections of growth hormone produce Pre-natal Developmental Healing (PDH). This unique action of growth hormone, proven in mature rabbits, does not involve bleeding, clot formation or the in-pouring of inflammatory cells. This healing is scar-free and produces no fibroartilage. The cascade of PDH first involves the rejuvenation of mature subchondral arteries to form fetal fenestrated capillaries which produce autologous stem cells. These are the same vessels which produce the fetal cartilage skeleton in utero. These stem cells are signaled to form chondrocytes and by the completion of the PDH cascade real articular cartilage with vertical parallel columns of chondrocytes form with arcades at the surface and 100 per cent bonding to host bone. This method is the first to regrow real articular cartilage.
Method: Sixty-six adults with advanced osteoarthritis of the knee were treated with intra-articular injections of Human Growth Hormone (HGH). One-third required arthroscopic debridement and abrasion of eburnated bone on the condyles.
Each knee received from eight to fifteen weekly injections of Human Growth Hormone (HGH). Dosage varied according to the size of the knee. All patients were required to be non weight bearing on the treated side for the duration of the treatment. Patients were required to perform simple exercises at home. Less than one-fourth required physiotherapy.
There were no complications or side effects from the surgery or the injections of human growth hormone (HGH)
Results: Eighty-seven percent of the patients had good to excellent results. Several before and after X-rays of the HGH treated knees will be presented. These X-rays demonstrate an increase of the joint spaces from 2 to 5 mm. Evaluation with the IKDC format will be presented in graph form. There were no infections, complications, side effects, deep vein thrombosis, pulmonary embolism or deaths. The patients who did not respond were no worse. Six per cent went on to have total knee arthroplasty (TKA). the remainder were undecided about having TKA.
Conclusions: A safe, cost-effective alternative to TKA is presented. There were no complications such as those which arise from TKA. The cost of treatment with HGH even including arthroscopic surgery is one-fourth that of TKA. And the additional costs of treating infected TKA are completely avoided. Many orthopedic surgeons are concerned that their livelihood will be adversely impacted by loss of TKA surgery; however, they should consider that there is no need for hospital rounds, or need to treat infections and other serious complications. Insurance companies and Medicare or other government insurance programs can save billions of dollars every year by avoiding costly TKA surgeries at $35,000.00 per TKA.
The author recommends that this HGH treatment, which he named IAGH, be the first choice for treating advanced osteoarthritis of the knee.

071

EFFECT OF ROSEMARY EXTRACT AND RELATED FLAVONOID CARNOSOL ON CHONDRO-PROTECTION AND ON THE BONE-CARTILAGE CROSSTALK

M.-N. Horcajada[1], C. Sanchez[2], F. Scalfo[1], L. Ameye[1], Y. Henrotin[2], E. Offord[1]
[1]Nestle, Lausanne, Switzerland; [2]Univ. of Liège, Liège, Belgium

Purpose: The aim of this work was to evaluate the effect of Rosemary extract (P31, Robertet, France) and one of its associated flavonoids, carnosol (Sigma, Buchs, Switzerland) on metabolic functions of chondrocytes and osteoblasts, as well as on the bone-cartilage crosstalk.
Methods: Content of carnosol in rosemary extract P31 was around 7%. Rosemary extract and carnosol efficacy were assessed at various concentrations in the different experiments: 3-25μg/ml and 6nM-30μM, respectively. Normal and Osteoarthritic (OA) human chondrocytes were cultured in alginate beads for 12 days in presence or absence of both compunds. Production of aggrecan (AGG), stromelysin (MMP-3), interleukin (IL)-6 and nitric oxide were analyzed. Isolated human osteoblasts from sclerotic (SC) or non sclerotic (NSC) subchondral bone were cultured for 3 days in presence or absence of both compounds and alkaline phosphatise (AP) activity, interleukin-6 and prostaglandin PGE2 levels were determined. Finally, subchondral osteoblasts coming from NSC or SC areas were incubated with rosemary extract or carnosol for 72h before coculture with OA chondrocytes in alginate beads. After 4 days of co-culture, we analyzed AGG content of the alginate beads, and chondrocytes gene expression of AGG, type II collagen, MMP-3, MMP-13 and osteopontin (OPN).
Results: In both OA and normal chondrocytes, AGG production was significantly increased with 9 μM carnosol. MMP-3 and nitric oxide production was significantly decreased by both compounds, in a dose-dependent manner, while only carnosol significantly decreased the cytokine IL-6 secretion. Regarding osteoblast cultures, AP activity was not affected in the

presence of carnosol in SC and NSC cultures and highly reduced in both cell cultures in presence of rosemary extract.Carnosol was able to reduce IL-6 production by both SC and NSC osteoblasts at all doses tested while rosemary extract was efficient only on osteoblasts from the NSC area. Both compounds were also able to significantly decrease PGE2 production by osteoblasts from both subchondral areas.
In the coculture experiments, carnosol pre-incubated in NSC and SC osteoblasts significantly increased AGG and significantly decreased MMP-3 and OPN gene expression by chondrocytes. Rosemary extract only significantly decreased MMP-3 expression by chondrocytes in presence of SC osteoblasts.
Conclusions: In our experimental conditions, we showed that carnosol, through anti-inflammatory mechanisms, was able to reduce cartilage matrix breakdown and enhance its formation, more consistently than rosemary extract, at physiological concentrations.

072

MESENCHYMAL STEM CELLS IN OA PATIENTS: DOWNREGULATION OF WNT SIGNALING PATHWAY AND MIR335

B. Fernandez-Gutierrez[1], E. Villafuertes[1], J. Hoyas[1], M. Gonzalez[2], P. Tornero-Esteban[1]
[1]Hosp. Clinico San Carlos, Madrid, Spain; [2]CNIC, Madrid, Spain

Purpose: Osteoarthritis (OA) is a disease characterized by progressive degeneration of articular cartilage and bone. Homeostasis of the articular tissues depends largely on the ability of self-renewal and differentiation of mesenchymal stem cells (MSCs) into different cell types of mesodermal lineage. This process is mediated by activation and suppression of different genes controlling post-transcriptional regulation of gene expression. miRNAs (short 20-24 nt non-coding RNAs) are key molecules affecting both the stability and translation of mRNAs. The expression of miR335 in control Bone Marrow-MSCs (BM-MSCs) has been previously connected with the canonical Wnt signaling pathway. According to these evidences, our purpose is to study, in BM-MSCs from OA patients, the canonical Wnt pathway and the expression of miR335.
Methods: Eight OA patients and eight controls were included. BM-MSCs from OA patients were obtained at the time of total joint replacement surgery of hip OA. BM-MSCs from controls were obtained at the time of surgery of subcapital hip fracture without OA signs and without osteoporosis.
Cells were isolated and expanded until the third passage. RNAs were extracted to perform comparative gene expression profiling using the Agilent 4×44 whole-genome expression array and the Agilent Human microRNA. After data filtering, background correction and, normalization, differentially expressed genes at $p<0.05$ level of significance showing more than, or less than, two-fold differences, were eligible. To determine miRNA expressions, RNA samples from five patients and five controls were hibridized and analyzed using the Microarray v2.0 (G4470B, Agilent). MEST gene, that controls miR335 expression, was analyzed in the Agilent 4×44 whole-genome expression array and validated by quantitative PCR (qPCR).
Results: Wnt pathway was clearly defective in MSCs from OA origin. Major differences showed a significant downregulation of 11 genes related to the Wnt pathway, these include CCND2, CSNK2A1, DVL1, DVL3, FZD3, LRP6, NLK, PPP3CC, SENP2, SFRP2 and WNT4. In addition, in all samples miR335 expression levels were diminished around 50% in OA patients compared to expression levels found in controls.
MEST gene was clearly downregulated in the Agilent 4×44 whole-genome expression array and this result is concordant with MEST qPCR results.
Conclusions: Our results suggest that expression of miR335 in MSCs is connected with Wnt signaling pathway also in OA patients. We hypothesize that the diminished miR335 expression and Wnt signalling pathway in OA could be a part of the altered function of BM-MSCs in OA patients.

073

MIR-7 AND MIR-130B ARE DIFFERENTIALLY REGULATED DURING MESENCHYMAL STEM CELL COMMITMENT

G. Palmer[1], L. Danielson[2], M. Attur[1], S.B. Abramson[1], E. Hernando[2]
[1]NYUHosp. for Joint Diseases, New York, NY; [2]NYU Langone Med. Ctr., New York, NY

Purpose: Stem cell-based therapies aimed at introducing progenitor cells into cartilage lesions hold great promise for the restoration of damaged articular surfaces following joint injury or osteoarthritis. Key to the generation of a functional repair tissue is the controlled differentiation into the desired phenotype. To this end microRNAs (miRNAs) may be important molecules that regulate this process. By acting as transcriptional repressors, their modulation during differentiation may enable commitment to a specific lineage by suppressing the expression of other lineage markers. In this study we profiled Mesenchymal Stem Cells (MSCs) for miRNA expression following induction into the chondrocyte (C), osteoblast (O) and smooth muscle (SM) lineages.
Methods: *Cell culture:* Human bone marrow derived MSCs were obtained from NIH or from the discarded hips of patients undergoing joint replacement surgery. *Differentiation:* SM differentiation was induced by treating monolayer cultures with 1 μM thromboxane-A2 [DP1] in the presence of 0.25% serum. C differentiation was induced by seeding MSCs in aggregate cultures in the presence of 1% ITS, dexamethasone (10^{-7} M) and TGF-β1 (10 ng/ml). O differentiation was induced by treatment of monolayer cultures with dexamethasone (10^{-7} M), ascorbate (37.5 μg/ml) and beta-glycerolphosphate (10 mM) in the presence of 10% serum. *miRNA profiling:* At various timepoints after induction, miRNA was extracted for analysis. miRNA profiling was performed by microarray (Agilent) or qPCR based assay (SA Biosciences). In all cases differentiation was confirmed by qPCR of lineage specific markers and histology.
Results: Among 376 miRNA probes, we noted differential regulation of two miRNAs among O, SM and C lineages. Following SM and C differentiation, miR-7 expression was down-regulated up to 6.9-fold and 3-fold respectively. Conversely, during O differentiation, its expression was induced approximately 7-fold. Analysis of theoretical mRNA targets using TargetScan online software (www.targetscan.org) identified conserved sites in several genes associated with chondrocyte and myoblast lineages. Putative chondrogenic targets were found to include COL2A1, IGFR1, and GDF5, while potential smooth muscle modulators included EGFR1, PIK3CD, IRS1/IRS2, KLF4, CNN3 and IGF1R. Following a similar trend to miR-7, miR-130b was down-regulated up to 3.2-fold and 3.1-fold in C and SM differentiation respectively, while O differentiation induced its expression 2-fold. TargetScan analysis identified putative chondrogenic targets, TGF-BRII, Sox5, BMP-2 and IGF1; Potential smooth muscle regulators included ESR1, TGF-BRII, MBLN1, TGFBR1 and IGF2BP1. Together these observations suggest that miR-7 and miR-130b act to negatively regulate myogenic and chondrogenic cell fates via regulation of lineage specific genes.
Conclusion: Our findings suggest that miR-7 and miR-130b, via the targeting of lineage specific molecules, regulate cell fate in adult human MSCs by inhibiting smooth muscle and chondrocyte differentiation, thereby promoting 'default' differentiation into the osteoblast lineage.
[DP1]0.25% FBS 24 hours prior to addition of 1.0μM of the TxA2 chemical analog U46619

074

BOVINE PRIMARY CHONDROCYTES STIMULATE CARTILAGINOUS MATRIX PRODUCTION BY HUMAN EMBRYONIC STEM CELLS

N. Ahmed[1,2], D.W. Taylor[1,2], G. Keller[3], M.D. Grynpas[1,2], R. Kandel[1,2]
[1]Mount Sinai Hosp., Toronto, ON, Canada; [2]CIHR-BioEngineering of Skeletal Tissues Team, Toronto, ON, Canada; [3]McEwen Ctr. for Regenerative Med., Univ. Hlth.Network, Univ. of Toronto, Toronto, ON, Canada

Purpose: A requirement of *in vitro* tissue generation for cartilage repair is sufficient number of cells which is difficult to attain from sparsely cellular cartilage tissue. Thus proper selection of cell source is an impending problem for cartilage tissue engineering. Optional cell types are chondrocytes, marrow progenitor cells and embryonic stem cells. Use of human embryonic stem cells (hES) for cartilage tissue engineering is still in its infancy mainly because the existing techniques do not render functionally stable cartilage tissue. To push hES cells towards a specific lineage cues are required from growth factors, media and culture conditions. Previously we have shown that co-culture of dedifferentiated chondrocytes with small numbers of primary chondrocytes induces redifferentiation in passaged dedifferentiated cells. In this study we show for the first time a novel culture approach where hES cells when co-cultured with bovine primary chondrocytes (bP0) exhibit the ability to deposit functional three-dimensional cartilaginous matrix.
Methods: *Cell preparation:* Bovine articular cartilage (6-9 months old) was harvested from metacarpo-phalangeal joints and chondrocytes were isolated by sequential enzymatic digestion. GFP labeled-human embryonic